A STUDY OF THE NUMBER OF GENES FOR LEAF RUST REACTION DIFFERENTIATING CERTAIN WHEAT VARIETIES

by

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INTRODUCTION

Wheat leaf rust, Puccinia triticina Eriksa, may be partially controlled by such cultural measures as change in date of sowing and fertilization. These are, however, only adjuncts to the fundamental solution of the problem, the breeding of resistant varieties. To plan and carry out a breeding program designed to produce resistant varieties a knowledge of the genetics of rust reaction is essential to make most rapid progress. Relatively little is known about the genetics or the nature of leaf rust reaction. Before complete control of the disease is attained by the production of resistant varieties, the genetics of the host as well as the nature of leaf rust reaction must be more fully understood.

Leaf rust, one of the most destructive diseases of wheat in Kansas, is prevalent during most seasons and causes losses in the forms of reduced pasturage in the fall or yield of grain the following summer. A genetic study, with the purpose of determining the inheritance of rust reaction in crosses among the eight leaf rust differential varieties and Pawnee, is being conducted at Kansas State College. The inheritance of rust reaction in the seven wheat crosses reported in this thesis is only a part of the program.

REVIEW OF LITERATURE

Nature of Leaf Rust Reaction

Rust reaction may be classified into two general response types, resistance and susceptibility. The inherent properties of a plant, functional, morphological, and physiological, which inhibit or allow the development and sporulation of the rust, determine whether it is resistant or susceptible.

Chester (1946) cited studies of various investigators in which they attempted to explain resistance in terms of such properties and constituents of cells as pH, osmotic pressure, aromatic compounds, and enzymes. Correlations have been obtained between resistance and content of phenolic compounds and gums. Such compounds, according to Chester, may mark off broad categories of resistance and susceptibility between species, but cannot explain physiologic specialization within a species. Chester stated that Gassner's hypothesis of dependence of resistance on specific proteins and antibody reactions seemed to be the most plausible and made progress toward explaining obligate parasitism.

Chester also presented a hypothesis of dependence of resistance and susceptibility on certain nutritional relationships between the host and parasite. In both resistant and susceptible plants, the fungus invades the host cells, but in resistant plants the cells of the host and fungus die. Chester believes that the fungus cells die as a result of starvation, the wheat plant being unable to supply specific nutrients needed by the parasite. Since the fungus is intracellular, its degenerate products kill the host

cells, causing the necrotic flecking observed in resistant reactions.

In higher plants resistance to wheat leaf rust is common. Susceptibility is rare, being confined to varieties of Triticum and to species of Thalictrum and Isopyron, alternate hosts. Since susceptibility is rare, Chester suggested that the key to varietal resistance in Triticum lies in determining the cause of susceptibility. He suggested that the cause of susceptibility may be certain proteins peculiar to species and varieties of Triticum.

Allen (1923) made critical cytological studies of the relation of certain races of wheat stem rust to resistant and susceptible hosts. Baart, a variety susceptible to races 3 and 19; Kanred, a variety susceptible to race 3 and highly resistant to race 19; and Mindum, which is immune to race 3 and susceptible to race 19, were studied comparatively. Race 3 formed normal haustoria in susceptible Baart and Kanred. The host cells proximal to the fungus showed increased metabolic activity, while the host nuclei increased in volume. Plastids decreased in size, but increased metabolic activity appeared to check this effect. The host nuclei eventually collapsed. Race 3 also formed haustoria in immune Mindum. In this case, the living contents of the host cells flowed rapidly to the haustoria and condensed around them. The protoplasm of the intercellular hyphae moved to the haustoria, as the haustoria formed and the host cells collapsed. Death of the haustoria followed collapse of the host cell. The fungus was thus impoverished, while the surrounding host cells became plasmolyzed. Essentially the same reaction was observed when resis-

tant Kanred was inoculated with race 19.

A more or less congenial relation exists between a susceptible host and the parasite during the initial states of infection; whereas this is not true in the case of a resistant host. According to Walker (1950), it is still a matter of conjecture whether the fungus produces a material toxic to the host cell or whether something in the host cell is toxic to the fungus. Walker also stated that the approach to the nature of disease reaction on the basis of presence or lack of nutrients essential to the pathogen deserves much more consideration than it has been given in the past.

Wheat seedlings, susceptible to leaf rust, may become resistant as they become older. Johnston (1937) has called this "the maturative type of resistance" which is now referred to as the mature or adult plant resistance. This type of resistance may appear very early in the development of the wheat plant, the second, third, and fourth leaves showing resistance as compared with the primary leaf (Johnston and Mains 1932). In contrast there are no clear cut cases in which varieties resistant to leaf rust as seedlings become susceptible as mature plants, a resistant reaction to a given race on the primary leaf being a good index of rust reaction to the same race throughout the entire life of the plant.

Inheritance of Leaf Rust Reaction

The literature on inheritance studies with wheat leaf rust are presented in Table 1. Chester (1946) reviewed the literature

up to 1939. In all crosses studied varieties have been found to be differentiated by one or two genes pairs.

Table 1. Inheritance of resistant reactions to wheat leaf rust.

Cross	Reference
Monohybrid Dominant	
Malakof (R)* x several (S)** varieties	(Mains et al., 1926)
Malakof (R) x C.I. 3778, Race 12	"
Norka x C.I. 3756, Races 5, 12	"
Webster (R) in varicus crosses	(Cited by Chester, 1946)
Rumpaus n. Schlanstedter x Normandie (R)	"
1014 x Normandie (R), Race 15	"
Webster x Mediterranean	(Mains, 1926)
Cannberra x Thew (R) Austr. 1	(Waterhouse, 1930)
Riverina x Thew (R) Austr. 1	"
Gluyas x Thew (R) x Federation Austr. 1	"
Japanese bearded (R) x Federation Austr. 1	"
Norka (R) x Ceres, Race 3	(Mains, 1934)
Vulg. 037 x Ukrainka, Poltavka	(Leighty, 1929)
Hope (R) x Leap's Prolific	(Adams, 1939)
Hope (R) x Fulcaster	"
Hope (R) x Purplestraw	"
Peragis x Normandie (R), Race 15	(Cited by Chester, 1946)
Blausamtiger Kolbers x Peragis (R), Race 15	"
Malakof x Susceptible wheats	(Leighty, 1926)
Malakof x several vars., Race 12, 3	(Leighty, 1929)
(H44-Reward x Baringa) x (Hard Federation x Dicklow)	(Wells and Swenson, 1944)

Table 1. (Con't.)

Cross	Reference
Monohybrid Dominant	
Malakof x Democrat, Races 5, 58	(Woodward, 1950)
Lee (R) x Mida, Race 5	(Wu and Ausemus, 1953)
Monohybrid Recessive	
Malakof x C.I. 3778 (R), Race 5	(Mains et al., 1926)
Kanred x Fulcaster	(Cited by Chester, 1946)
Harvest Queen x Fulcaster	"
Harvest Queen x Kanred	"
Ardito (R) x San Martin	"
Ardito (R) x various S varieties	"
Varonne (R) x various S varieties	"
Normandie (R) x various S varieties	"
Dinkel x emmers (usually)	"
Durum x vulgare	"
Turgidum x compactum	"
Polonicum x spelta	"
Malakof x C.I. 3778 (R), Race 5	(Leighty, 1929)
Susceptible vars. x Democrat, Mediterranean 35.27	(Cited by Chester, 1946)
Kanred x Fulcaster	"
Hope (R) x Ukrainka	"
Marquis x Ukrainka	"
Odessa 0719 x Mediterranean (R)	"
Chino 166 (R) x Lin Calel	"

Table 1. (Con't)

Cross	Reference
Monohybrid Recessive	
Kanred x several susceptible varieties	(Mains et al., 1926)
(Mediterranean-Hope x Pawnee) x Comanche, Race 9	(Schlehuber, 1950)
(Oro x Mediterranean x Hope) x Comanche, Race 9	"
(Kawvale - Marquillo x Kawvale-Tenmarq) x Cheyenne, Race 9	"
(Kawvale - Marquillo x Kawvale-Tenmarq) x Comanche, Race 9	"
Wabash x Michigan Amber, Races 9, 31, 65, 78, 79, 80, 101, 110	(Caldwell, et al., 1947)
Lee x Mida, Race 126	(Wu and Ausemus, 1953)
Monohybrid Incomplete Dominance	
Kanred x several S varieties	(Mains, et al., 1926)
Malakof (R) x several S varieties	"
Malakof x Webster (R)	"
Fulcaster (R) x Kanred	"
Vulgare Chinese x dicoccum (R)	(Guard, 1938)
Malakof x R varieties	(Leighty, 1926)
037 x Cooperatorka	(Cited by Chester,1946)
Marquis x Lutescens 0329	"
Pawnee x RedChief, Race 9	(Penny, 1947)
Dihybrid Dominant	
Norka x C.I. 3756	(Mains et al., 1926)
12H x 38 MA (R)	(Cited by Chester,1946)

Table 1. (Concl.)

Cross	Reference
Complimentary Dominant Factors	
Pawnee x Chinese, Race 9	(Heyne and Livers, 1953)
Dihybrid Recessive	
Vulgare 037 x Minhardi	(Cited by Chester,1946)
Vulgare 037 x Belaya Tserkov 6182	"
Malakof x Democrat, Race 9	(Woodward, 1950)
Dihybrid Incomplete Dominance	
Lee (R) x Mida (mixture of races)	(Wu and Ausemus,1953)
Transgressive Segregation for Resistance	
Thatcher x Triumfo (mixture of races)	(Swenson et al.,1947)
Oro x Tenmarq (mixture of races)	(Wismer,1934)
Linkage Between Reactions to Races	
Lee x Mida Races 5 and 126, 21± 2.73	(Wu and Ausemus, 1953)

*(R) Resistant
**S Susceptible

METHODS AND MATERIALS

The mode of inheritance of seedling reaction to physiologic races 5, 9, 15, 19, and 58 of wheat leaf rust was studied in seven crosses, using F_3 lines and bulk F_3 populations. Seedling plants were classified by the rust reaction on the primary leaf, according to standard reaction types used in identifying physiologic races. These standard reaction types were described by Mains and Jackson (1926) as follows:

0. Highly resistant; no uredinia formed; flecked.
1. Very resistant; more or less flecked; accompanied by few small uredinia.
2. Moderately resistant; uredinia fairly abundant and of moderate size, accompanied by necrosis.
3. Moderately susceptible, uredinia fairly abundant and of moderate size, sometimes chlorosis.
4. Very susceptible; uredinia abundant, sometimes in more or less pronounced green islands.

In addition to the types described above, two reaction types, considered to be in the resistant category and designated as X and Y, were used. An X reaction, described by Johnston and Mains (1932), commonly observed in hybrids, is characterized 0 and 4 reaction types occurring at random on the same leaf. Abundant pustules of the 4 type on the upper one-third of the leaf grading down to a 0 type reaction at the base of the leaf characterizes the Y reaction.

The parental varieties and their reactions to the races used

in the study are presented in Table 2.

Table 2. Parental varieties and their reactions to the five physiologic races of leaf rust.

Variety	Physiologic Race				
	5	9	15	19	58
Malakof CI 4898	4	4	0	4	0
Carina CI 3756	0	1-2	0	4	2
Brevit CI 3778	0-1	1-2	0-1	2	3-4
Webster CI 3780	0-1	4	0	4	1
Loros CI 3779	0-1	4	0-1	4	3
Mediterranean CI 3332	4	0-1	4	0	4
Hussar CI 4843	2+	1-2+	0-1	1	2
Pawnee CI 11669	4	0-1	4	0-1	4

Seedlings were tested to only one physiologic race at a time. Purity of race was maintained by keeping each race isolated in a greenhouse section. Racial purity was checked by growing at least four differential varieties in each test. After completion of each race study, the greenhouse was thoroughly cleaned before another race was introduced.

Fresh inoculum for each seedling test was provided by growing and inoculating six 3-inch pots of the susceptible wheat variety, Cheyenne. Inoculations, using pure race cultures, were made ten days following each planting. The same day Cheyenne was inoculated, twenty-five to fifty seeds of each F_3 line together with the parents and at least four differential varieties were planted

in one-hundred 3-inch pots. In order to identify its progeny, the seed from an individual F_2 plant was assigned a number which was then used throughout the tests. Ten days following planting, the seedlings were inoculated by brushing them with infected Cheyenne plants and incubated in a portable, cloth moist-chamber for twelve hours.

Readings to determine the breeding behavior of the F_3 lines were taken approximately ten days after inoculation. The lines were classified homozygous resistant, homozygous susceptible or segregating for rust reaction. In homozygous lines a single reading was taken, while in segregating lines individual plants were classified as to reaction type.

The phenotypic expectation for the general case in segregating F_3 lines with duplicate genes involved, where p and 1-p are the recombination values for repulsion and coupling respectively, were calculated in the following manner.

Genotype	Proportion of Segregating Genotypes	Manner of Segregation AB	:	ab
AaBb	$(1-2p+2p^2)$	$\frac{(4-p^2}{4}$	:	$\frac{p^2)}{4}$
Aabb or aaBb	$2p\ (1-p)$	$\frac{(3}{4}$	:	$\frac{1)}{4}$
		$\frac{4-2p+p^2+2p^3-2p^4}{4}$	:	$\frac{2p-p^2-2p^3+2p^4}{4}$

Thus, the expectation for the ab class is $(1-2p+2p^2)\ \frac{(p^2)}{4} + \frac{2p(1-p)}{4} = \frac{2p-p^2-2p^3+2p^4}{4}$. When p is 0.5, the AB and ab classes occur in a ratio of 0.844:0.156 or 27:5. Similarly, the phenotypic or genetypic expectation for segregating F_3 lines in any

cases where two genes were involved were calculated by the same method.

Phenotypic expectation in the bulk F_3 populations for three independent factors was calculated as follows:

(5A:3a)(5B:3b)(5C:3c) = 125ABC:75ABc:75AbC:75aBC:45Abc:
45aBc:45abC:27abc.

The breeding behavior of F_3 lines, i.e., whether they are homozygous or heterozygous, can be classified with accuracy and precision. Classification of individual plants in segregating lines was, however, subject to greater error, due to variation in environmental conditions in the greenhouse affecting the development of both the host and parasite.

In certain cases, the observed fraction of resistant plants in segregating lines was far below expectation, suggesting that gene penetrance was incomplete. Penetrance of genes for resistance was defined as Penetrance = $\frac{1}{T}\left[O \pm t_{.05}\sqrt{\frac{O(1-O)}{n}}\right]$, where T = theoretical fraction of resistant plants, O = observed fraction of resistant plants, n = number of observations, and $t_{.05}$ = 5% t-value for degrees of freedom equal to infinity.

The recombination value for the case of linked duplicate genes in coupling was estimated by the method of maximum likelihood. The formulae were derived by Dr. Fryer, Statistics Department, Kansas State College.

$$\hat{p} = 1 - \sqrt{\frac{2n_2}{n_1+n_2}}$$

$$\text{S. E. } \hat{p} = \sqrt{\frac{2n_1}{n(n_1 + n_2)}}$$

n_1 = number of lines breeding true for the AB class.

n_2 = number of lines breeding true for the ab class.

n = number of observations.

EXPERIMENTAL RESULTS

Seedling Reaction to Five Races of Leaf Rust

The breeding behavior of the F_3 lines and the phenotypic classification of the segregating F_3 lines and bulk F_3 populations are presented in Tables 3 and 4.

Mediterranean was resistant to race 9 and susceptible to races 5, 15, and 58. Webster was resistant to races 5, 15, and 58, but susceptible to race 9. These varieties were differentiated by a single gene pair for reaction to race 9 and for reaction to races 5, 15, and 58 as supported by the data (Tables 3 and 4). The resistant reaction of Webster to races 5, 15, and 58 was completely dominant to the Mediterranean susceptible reaction. The breeding behavior of the F_3 lines tested to races 5, 15, and 58 was the same, indicating that the resistance of Webster to these races was due to the same gene. The resistant reaction of Mediterranean to race 9 was incompletely dominant to the reaction of Webster. Heterozygotes were presumably of the X type reaction, but could not be distinguished accurately from homozygous resistant plants. If readings were taken before the rust was sporulating freely, the X reaction could not be separated from a 4 type as indicated by the penetrance value, $0.942 \pm .01$ (Table 4). Penetrance was complete if readings were taken when the rust was fully developed.

Carina and Hussar were both resistant to races 5, 9, 15, and 58. The Hussar reaction to these races varied from 2-2+. The

Carina reaction was 0 to races 5 and 15, and 1-2 to races 9 and 58. Transgressive segregation for susceptibility to all four races was obtained. The data support the hypothesis that two different factors, segregating independently in the F_2, were responsible for the resistance of these varieties to the four races. With few exceptions, probably due to errors in classification, all lines breeding true for resistance and segregating 0-2 to races 5 and 15 bred true for resistance to race 58, suggesting that the same genes were involved in the reaction to these races. The Carina reaction to races 5 and 15 was epistatic to the Hussar reaction when the Carina genes for resistance were homozygous, as shown by the small departure from the 1:4 ratio (Table 4). The Carina and Hussar genes behaved as independent-dominant duplicate factors when the F_3 lines were tested to races 9 and 58. Penetrance of Carina genes for resistance was $0.897 \pm .012$ and $0.742 \pm .014$ for races 9 and 58 respectively (Table 4).

Pawnee was susceptible to races 5, 15 and 58. The Carina resistant reaction to these races in the cross, Pawnee x Carina, was dominant to susceptibility. Both parents were extremely variable in their reaction to race 58 but gave stable reactions to races 5 and 15. Each line breeding true or segregating to races 5 and 15 behaved in the same manner when tested to race 58, suggesting that the same genes were responsible for the reactions to all three races. Linked duplicate genes in the coupling phase with 16.3 ± 9.3 percent crossing over differentiated the reactions of these varieties to the three races as evidenced by the data (Tables 3 and 4). Apparently Carina was composed of

Table 3. Classification of leaf rust reaction of F_3 lines with hypotheses and Chi-square test of goodness of fit.

Cross	Physiologic Race	Leaf Rust Reaction of F_3 Lines			Hypothesis	X^2	P lies between
		0-1*	Seg.	4			
Webster x Mediterranean	9	50	90	53	1:2:1	0.968	0.7 -0.5
	5	46	96	47	1:2:1	0.070	0.98-0.95
	15	40	93	48	1:2:1	0.728	0.5 -0.3
	58	34	87	39	1:2:1	0.728	0.5 -0.3
Carina x Hussar	9	1-2+* 62	Seg. 72	4 7	7:8:1	0.355	0.9 -0.8
	5	0 34	0-2-4# 39	0-2# 17	4:4:2:2:2:1:1	3.504	0.8 -0.7
		0-4# 11	2-4# 20	2/9 4/9			
	15	0 34	0-2-4# 41	0-2# 21	4:4:2:2:2:1:1	9.030	0.2 -0.1
		0-4# 7	2-4# 18	2/6 4/9			
	58	2-2+* 58	Seg. 62	4/9	7:8:1	0.249	0.7 -0.5
Carina x Pawnee	5	0-1* 51	Seg. 72	4 27	Linked duplicate genes 16.3 ±9.3 crossing over	0.247	0.9 -0.8
	15	50	72	27	"	0.168	0.95-0.9
	58	2-2+* 50	Seg. 67	4 25	"	0.555	0.8 -0.7

Table 3. (Concl.)

Cross	Physiologic Race	Leaf Rust Reaction of F_3 Lines			Hypothesis	X^2	P lies between
Brevit x Hussar	9	$\frac{2\text{-}2+*}{52}$	$\frac{\text{Seg.}}{83}$	$\frac{4}{8}$	7:8:1	3.733	0.2 -0.1
	15	$\frac{0\text{-}1*}{63}$	$\frac{\text{Seg.}}{64}$	$\frac{4}{13}$	7:8:1	2.628	0.3 -0.2
Carina x Malakof	5	$\frac{0}{95}$	$\frac{\text{Seg.}}{115}$	$\frac{4}{138}$	4:6:6	2.948	0.3 -0.2
Loros x Pawnee	5	$\frac{0\text{-}1*}{39}$	$\frac{\text{Seg.}}{101}$	$\frac{4}{61}$	1:2:1	4.822	0.1 -0.05
Webster x Pawnee	5	$\frac{0}{58}$	$\frac{\text{Seg.}}{120}$	$\frac{4}{68}$	1:2:1	0.359	0.7 -0.5

* Range of reaction
\# Manner of segregation
Seg. Segregating

Table 4. Phenotypic classification of leaf rust reaction of individual plants in segregating lines and bulk F_3 populations with hypotheses and Chi-square test of goodness of fit.

Cross	Physiologic Race	Phenotypic Classification:		Hypothesis	X^2	P lies between:	Penetrance
Webster x Mediterranean	9	0-1* / 1380	4 / 574	3:1	20.026	<0.01	0.942±.01
	5	1654	585	3:1	1.459	0.3-0.2	100
	15	2876	950	3:1	0.051	0.9-0.8	100
	58	2978	1087	3:1	6.475	0.02-0.01	100
Carina x Hussar	9	1-2+* / 1799	4 / 577	27:5	134.882	<0.01	0.879±.012
	5	0 / 427	2-4* / 1772	1:4	0.430	0.7-0.5	100
	15	790	2956	1:4	2.704	0.2-0.1	100
	58	2-2* / 2272	4 / 1391	27:5	1386.123	<0.01	0.742±.014
Carina x Pawnee	5	0-1* / 1069	4 / 375	Linked duplicate genes 16.3%±9.3 crossing over	37.428	<0.01	0.92±.014
	15	2546	672	"	3.549	0.1-0.5	100
	58	2-2+* / 1161	4 / 1416	"	>100	<0.01	0.56±.019

Table 4. (Con't.)

Cross	Physiologic Race	Phenotypic Classification		Hypothesis	X^2	P lies between	Penetrance
Brevit x Hussar	9	1-2*/2758	4/731	27:5	74.683	< 0.01	0.937±.008
	15	1924	1082	27:5	758.894	< 0.01	0.759±.066
Carina x Malakof	5	0/2916	4/972	3:1	1.233	0.3-0.2	100
Loros x Pawnee	5	0-1*/2382	4/776	3:1	0.285	0.7-0.5	100
Webster x Pawnee	5	0/2786	4/974	3:1	1.640	0.3-0.2	100
		Phenotypic classification of F_3 populations					
Carina x Malakof	5	0/2375	4/1198	3.35:1.77	1.665	0.2-0.1	100
	15	436	245	3.17:1.95	1.213	0.3-0.2	100
	19	0/1209	Y/902	4/812	1.65:1.25:2.22	0.686	0.5-0.3	100
Webster x Mediterranean	9	0-x*/1405	4/812	5:3	0.686	0.5-0.3	100
	5	0/1271	4/665	5:3	8.067	< 0.01	100

Table 4. (Concl.)

Cross	Physiologic Race	Phenotypic Classification		Hypothesis	X^2	P lies between:	Penetrance
Loros x Pawnee	5	0-1* / 1562	4 / 1022	5:3	4.551	0.05-0.02	100
Webster x Pawnee	5	0 / 1164	4 / 570	5:3	0.693	0.5-0.3	100

* Range of reaction.

different genotypes with respect to rust reaction, since independent segregation was observed in the Carina x Hussar cross tested to races 5, 15, and 58. Estimated penetrance of genes for resistance to 5 and 58 was 0.92± .014 and 0.56± .019 respectively (Table 4).

Both Brevit and Hussar were resistant to races 9 and 15. Transgressive segregation for susceptibility to both races was obtained, indicating Brevit and Hussar possessed different dominant factors for resistance to each race. These factors segregated independently in the F_2 as shown by the nonsignificant deviation from the 7:8:1 ratios (Table 3). Penetrance was estimated as 0.937± .008 for race 9 and 0.759± .066 for race 15 (Table 4).

Malakof was susceptible and Carina was resistant to race 5. Resistance was completely dominant in this cross, being conditioned by a single gene. However, the number of homozygous susceptible and segregating F_3 lines were far from expectation in a monohybrid ratio. It appeared as if the gene for resistance and a sticky gene, causing non-disjunction at anaphase I of meiosis if homozygous and hypostatic to homozygous genes for resistance, were located on the same chromosome at least fifty crossover units apart. Parental and F_2 genotypes together with their breeding behavior in the F_3 are as follows:

Parents: Carina AABB x Hussar aabb

F_2 Genotype	Breeding Behavior in F_3
1 AABB	Breeds true; 0
2 AaBB	Breeds true phenotypically; 4
2 AABb	Breeds true phenotypically; 0

4 AaBb	Segregates; 10=0, 6=4.
1 AAbb	Breeds true; 0
2 Aabb	Segregates; 3=0, 1=4
1 aaBB	Breeds true; 4
2 aaBb	Breeds true phenotypically; 4
1 aabb	Breeds true; 4

If it is assumed that A is the gene for resistance, under the above hypothesis all AA--genotypes breed true for resistance. Non-disjunction seemed to occur in AaBB and aaBB genotypes. Apparently chromosomes carrying the B factor in the homozygous condition were lost during meiosis, resulting in a high proportion of nullisomics which were susceptible because the A gene was not present. Chi-square tests show the data closely fit the expected ratios of 4:6:6 and 3:1 for the breeding behavior of the F_3 lines and in the segregating lines, P lying between 0.3 and 0.2 in both cases (Tables 3 and 4). Resistant and susceptible plants occurred in a ratio of about 2:1 in the bulk F_3 population. This 2:1 ratio is not explainable with the above hypothesis, but may be explained on a three factor basis. With a three factor hypothesis phenotypes ABC, AbC, Abc, abC, and Abc are resistant; phenotypes aBC, ABc and abc are susceptible, and occur in a ratio of 3.35:1.17 (Table 4). The Chi-square test for goodness of fit was good, P lying between 0.2 and 0.1. It seems paradoxical that the breeding behavior of the F_3 lines and the segregation observed in the bulk F_3 population cannot be explained with the same hypothesis. The peculiar behavior of the Carina x Malakof cross, when tested to race 5, remains an enigma which

will probably be clarified only by cytological investigations.

Carina and Malakof were both highly resistant to race 15 and susceptible to race 19. Transgressive segregation for susceptibility to race 15 and high resistance to race 19 was obtained in bulk F_3 populations. Three factors appeared to be involved in both cases. The segregation in the bulk F_3 population tested to race 15 may be explained by the following factorial hypothesis.

Parents: Carina (abc) x Malakof (ABC).

Phenotype	Reaction to Race 15
ABC	0
AbC	0
abC	0
aBc	0
abc	0
aBC	4
ABc	4
Abc	4

The Chi-square test indicates the observed numbers of resistant and susceptible plants do not deviate significantly from the expected ratio of 3:17:1.95 (Table 4). Transgressive segregation for resistance to race 19 was explainable with the following factorial hypothesis.

Parents: Carina (xyZ) x Malakof (XYz).

Phenotype	Reaction to Race 19
XYZ	Y
XyZ	0
Xyz	0

Phenotype	Reaction to Race 19
xYz	0
XYz	4
xYZ	4
xyZ	4
xyz	4

A fair fit to the expected ratio, 1.65-0:1.25-y:2.22-4 was obtained, P lying between 0.1 and 0.05 (Table 4).

Loros was resistant to race 5 and Pawnee susceptible. A single gene, as supported by the data in tables 3 and 4, differentiates these varieties with respect to reaction to race 5, resistance being incompletely dominant.

Pawnee was susceptible to race 5; Webster resistant. As indicated by the data (Tables 3 and 4) a single gene differentiates these varieties. The resistant reaction of Webster is also completely dominant in this cross.

Inheritance of Glume Color

Carina had brown glumes and Malakof white glumes. Three genes differentiated these varieties with respect to glume color. In this cross, brown glume color appeared to be dependent upon gene dosage, at least two dominant genes being required for color. Genotypes with six dominant genes were very dark brown. The following F_2 data support this hypothesis.

Dark Brown	Medium Brown	White
5	291	52

$x^2 = 0.171$, D. F. = 2, P = 0.05-0.9.

Loros had brown glumes and Pawnee white glumes. The F_2 was classified as follows as to glume color. Deviations from a 3:1 ratio are not significant, indicating a monofactorial basis of inheritance with brown dominant.

Brown	White
159	42

$x^2 = 1.195$, D. F. = 1, P = 0.3-0.2

Associations Between Characters

Chi-square tests for independence of inheritance between characters are presented in Table 5. In the Webster x Mediterranean cross, the reaction to race 9 and the reaction to races 5, 15, and 58 were inherited independently, the deviations from a two-factor genotypic ratio being nonsignificant. The reactions to race 9 and races 5, 15, and 58 in the Carina x Hussar cross, and the reactions to race 9 and 15 in the Brevit x Hussar cross were not inherited independently. Resistant reactions to all four races were definitely associated in these crosses. Unfortunately, the paucity of genetic data in these studies prohibits explaining these associations with factorial hypotheses. The Chi-square test for independence indicates reaction to race 5 and glume color was inherited independently in the Loros x Pawnee cross.

Table 5. Chi-square test for independence of inheritance between characters.

Cross	Characters	N	D.F.	X^2	P lies between
Webster x Mediterranean	Reaction to race 9 vs. Reaction to races 5, 15 and 58	189	8	7.200	0.5-0.3
Carina x Hussar	Reaction to race 9 vs. Reaction to races 5, 15, and 58	137	4	24.140	<0.01
Brevit x Hussar	Reaction to race 9 vs. Reaction to race 15	137	4	36.548	<0.01
Loros x Pawnee	Reaction to race 5 vs. Glume color	201	1	3.211	0.1-0.05

DISCUSSION OF EXPERIMENTAL RESULTS

Genetically speaking, a resistant reaction to leaf rust under particular environmental conditions may be due to the action of a single gene, as illustrated by the completely dominant reaction of Webster to races 5, 15 and 58 in the progeny of the Webster x Mediterranean cross; to intraallelic interaction, as illustrated by the progeny giving 0 type reactions in the Carina x Hussar cross; or to the additive effects of allelic genes as illustrated by the incompletely dominant reaction of Mediterranean to race 9. In the reaction of the Webster x Mediterranean cross to race 9, individuals homozygous for resistance genes displayed a higher type of resistance than heterozygotes. That resistance to leaf rust may also be due to both inter- and intraallelic interaction was suggested in the reaction of the Carina x Malakof to races 15 and 19. Resistance appeared to be due to either the interaction of dominant genes, XZ, or recessive genes, yz and xz. In the Carina x Malakof cross the phenotype, abc, was among those resistant to race 15. In the case of trihybrid recessive phenotypes, all genes must be in the homozygous condition to express resistance, indicating that both inter- and intraallelic interactions were involved in the resistant reaction. Similar genic relationships appeared to condition susceptibility. The evidence presented for the hypothesis explaining the reactions of the Carina x Malakof cross to races 15 and 19 was not conclusive, however, since the data were based entirely on the reaction of individual plants.

In all seven crosses studied, whenever both parents were resistant to a physiologic race, transgressive segregation for susceptibility was obtained, demonstrating that resistance to a race may be caused by at least two distinct genes. These genes, which appeared to be acting independently and were non-additive in their effects, were exemplified by the reaction of the Brevit x Hussar cross to race 9. Lines homozygous for two genes for resistance gave the same phenotypic reaction as lines homozygous for one gene, as indicated by the 7:8:1 ratio (Table 3). Any cumulative effects of genes for resistance were not detectable.

The occurrence of transgressive segregation for both resistance and susceptibility indicated that the varieties possessed genes for leaf rust reaction that were inhibited in their expression. The expression of these genes became manifest when combined in new combinations by segregation and recombination. The fact that different combinations of genes, as in the reaction of the Carina x Malakof cross to races 15 and 19, gave the same phenotypic expression suggested there may be many genes for leaf rust reaction. Any two varieties probably possessed many genes for rust reaction, but were differentiated by one or two gene pairs as determined by genetic tests.

That a series of multiple alleles for leaf rust reaction may exist was suggested by the reactions of the Carina x Hussar and Carina x Malakof crosses to race 5. The resistant reaction of Carina was completely dominant in the Carina x Malakof cross. In the Carina x Hussar cross, it was necessary for the Carina genes for resistance to be homozygous in order for the Carina re-

action to be expressed. Phenotypically the resistant reaction of Carina was the same in both crosses. Two explanations were possible. Either alternative alleles for the 0 type reaction to race 5 occurred in the variety, Carina, or Hussar and Malakof carried different alleles for susceptibility. The fact that Carina was found to possess linked duplicate genes for resistance in Carina x Pawnee cross tested to race 5 strongly suggested that the variety, Carina, was composed of a series of genotypes in which alternative alleles for rust reaction existed.

The expression of leaf rust reaction was not only dependent upon the genetic constitution of the host but also upon a particular race of pathogen. For example, Webster gave a 0 type reaction with small necrotic flecks to races 5 and 15 and a 1 type reaction with very large necrotic areas to race 58, yet the same gene was responsible for resistance to all three races. Similarly the expression of the Carina genes for resistance was of the 0 type reaction to races 5 and 15 but of a 2 type to race 58.

If it were assumed that the host genes for leaf rust reaction were necessary for the production of specific nutrients essential for the growth of the pathogen and that physiologic races of rust have specific nutrient requirements determined by their genetic constitution, the behavior of wheat leaf rust on wheat seemed analogous to the behavior of certain mutant strains of Neurospora on minimal media as reported by Emerson (1952). Emerson cited instances in which the growth of mutant strains of Neurospora having specific nutrient requirements for particular amino acids was inhibited by the presence of other amino acids not ordinarily

interfering with growth. This inhibition of growth seemed comparable to the Y reaction of the Carina x Malakof cross to race 19. Phenotypes XYz and xYZ were among those which gave a susceptible reaction to race 19. The growth of the rust, apparently, was not inhibited when XYz or xYZ factors were present. On the other hand, the presence of XYZ factors seemed to inhibit the growth of the rust. Emerson cited other cases in which mutant strains of Neurospora would not grow unless supplied a specific nutrient. Such cases may be comparable to the Webster reaction to races 5, 15 and 58 in which a single completely dominant gene was responsible for resistance. Webster, evidently, could not supply essential factors for the growth of the rust.

Comparisons between leaf rust and Neurospora may be untenable, but superficial analogies as given above seemed to exist. Neurospora may be readily cultured on artificial media; whereas, the 2n stage of wheat leaf rust is an obligate parasite found only on wheat. Whether or not the host genes are factors influencing the nutrition of the pathogen can only be decided by further research.

SUMMARY

The inheritance of leaf rust reaction was studied in seven wheat crosses, Webster x Mediterranean, Carina x Hussar, Carina x Pawnee, Carina x Malakof, Brevit x Hussar, Loros x Pawnee, and Webster x Pawnee. With the exception of Pawnee, the parents comprise seven of the eight leaf rust differential varieties used in identifying physiologic races. Physiologic races 5, 9, 15, 19, and 58 were used in the study.

Webster had one dominant factor for resistance to races 5, 15, and 58. Mediterranean had one incompletely dominant factor for resistance to race 9. In the Webster x Mediterranean cross, resistance to race 9 and to races 5, 15, and 58 were inherited independently. The resistant reaction of Webster was also completely dominant and monofactorially inherited in the Webster x Pawnee cross tested to race 5. The behavior of Carina was inconsistent in the three crosses tested, suggesting the variety was composed of different genotypes for rust reaction. Carina and Hussar carried different genes for resistance to races 5, 9, 15, and 58. The Carina reaction to races 5 and 15 was epistatic to the Hussar reaction, when the Carina genes for resistance were homozygous. The reaction to race 9 and to races 5, 15 and 58, which appeared to be due to the same genes, were not independently inherited. Carina and Pawnee were differentiated by linked duplicate genes in coupling with 16.3±9.3 percent crossing over in their reactions to races 5, 15, and 58. The behavior of the Carina x Malakof cross tested to race 5 in which resistance ap-

peared to be conditioned by a single gene, could not be adequately explained. Three factors appeared to be involved in the transgressive segregation for susceptibility to race 15 and high resistance to race 9 in the Carina x Malakof cross tested to these races. Brevit and Hussar carried different genes for resistance to races 9 and 15. The reactions to races 9 and 15 were associated in inheritance in this cross. The resistance of Loros to race 5 was differentiated from the susceptibility of Pawnee by a single gene. The reaction of Loros was incompletely dominant.

The inheritance of glume color was studied in the Carina x Malakof and Loros x Pawnee crosses. Three genes differentiated Carina and Malakof with respect to glume color. Brown glume color was dependent upon at least two dominant genes. Gene dosage appeared to influence color intensity. The brown glume color of Loros was differentiated from the white glumes of Pawnee by a single dominant gene. Brown glume color and reaction to race 5 were inherited independently.

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A STUDY OF THE NUMBER OF GENES FOR LEAF RUST REACTION DIFFERENTIATING CERTAIN WHEAT VARIETIES

by

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To plan and carry out a breeding program designed to produce leaf rust resistant varieties a knowledge of the genetics of rust reaction is essential to make the most rapid progress. A study, with the purpose of determining the genetics of rust reaction in crosses between the eight leaf rust differential varieties and Pawnee, is being conducted at Kansas State College. Only seven of these crosses were reported on in this study.

The mode of inheritance of seedling reaction to leaf rust in the crosses, Webster x Mediterranean, Carina x Hussar, Carina x Pawnee, Carina x Malakof, Brevit x Hussar, Loros x Pawnee, and Webster x Pawnee, was studied using F_3 lines and bulk F_3 populations. Seedling plants were classified by the rust reaction on the primary leaf, according to standard reaction types used in identifying physiologic races. Physiologic races 5, 9, 15, 19, and 58 were used in this study.

Webster and Mediterranean were differentiated by a single gene in their reactions to races 5, 9, 15, and 58. The resistance of Webster to races 5, 15, and 58 was completely dominant, while the resistance of Mediterranean to race 9 was incompletely dominant. The reactions to races 5, 15, and 58, which were governed by the same genes in this cross, were inherited independently of the reaction to race 9. The resistant reaction of Webster was also completely dominant and monofactorially in the progeny of the Webster x Pawnee cross was tested to race 5.

Transgressive segregation for susceptibility was obtained in the Carina x Hussar cross tested to races 5, 9, 15, and 58. The Carina reaction to races 5 and 15 was epistatic to the resistant

reaction of Hussar when the Carina genes for resistance were in the homozygous condition. When tested to races 9 and 58, Carina and Hussar genes for resistance behaved as independent, dominant, duplicate factors in the F_2. The reactions to races 5, 15, and 58, which appeared to be due to the same genes, and the reactions to race 9 were associated in inheritance in this cross. The resistance of Carina to races 5, 15, and 58 was differentiated from the susceptibility of Pawnee by linked duplicate genes in coupling with 16.3 ± 9.3 percent crossing over.

The reaction of the Carina x Malakof cross to race 5 could not be adequately explained. Resistance seemed to be conditioned by a single completely dominant gene from Carina, but the number of segregating and homozygous susceptible lines were far from expectation in a monohybrid ratio. The inconsistent behavior of the Carina reaction in the three crosses suggested that the variety may be composed of different genotypes for rust reaction.

Carina and Malakof were resistant to race 15 and susceptible to race 19. Transgressive segregation for susceptibility to race 15 and high resistance to race 19 was obtained in bulk F_3 populations. Three factors appeared to be involved in both cases, with resistance or susceptibility dependent on specific combinations of genes.

The resistance of Brevit and Hussar to races 9 and 15 was due to separate genes, as indicated by the transgressive segregation for susceptibility to both races. These genes behaved as independent, dominant, duplicate factors in the F_2. The reactions to races 9 and 15 were not inherited independently in this cross.

The resistant reaction of Loros to race 5 was differentiated from the susceptibility of Pawnee by a single gene. The reaction of Loros was incompletely dominant.

The inheritance of glume color was studied in the Carina x Malakof and Loros x Pawnee crosses. Three genes differentiated Carina and Malakof with respect to brown glume color, the brown color of Carina being dependent upon the presence of at least two dominant genes. Gene dosage appeared to influence color intensity. The brown glume color of Loros was differentiated from the white glumes of Pawnee by a single dominant gene. Brown glume color and reaction to race 5 were inherited independently.